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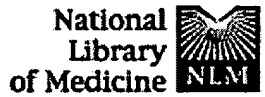

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1860

TRANSLATIONAL CONTROL MECHANISMS UTILIZED BY THE SEROTONIN 5HT2A RECEPTOR GENE K.Musser, B.Wilcox, and J.J.Jeffrey, Albany Medical College, Albany, NY 12208

Our laboratory has been investigating the rat serotonin 5HT2a receptor gene and its translational regulation. This gene contains an unusually long 5'-untranslated region (5'-UTR) of approximately 1100 nucleotides, two upstream open reading frames (uORFs), and thirteen upstream initiation codons (uAUGs). Chloramphenicol acetyltransferase (CAT) reporter gene constructs are utilized to study portions of the 5'-UTR of the 5HT2a receptor. Our initial studies indicate that one of the uORFs is capable of decreasing translational efficiency by approximately five fold. We are presently investigating several other potential regulatory regions of this 5'-UTR. In addition to these studies we are also investigating the possibility that this gene utilizes an alternate promoter to direct the transcription of a smaller mRNA containing less 5'-UTR. We have identified an alternate transcriptional start site through primer extension that is 420 nucleotides downstream of the start site for the full-length mRNA. Furthermore, in the rat 5HT2a gene we have identified potential promoter elements that are upstream of this region and Northern analysis reveals two mRNAs for this gene, one full length and one that is 400-500 nucleotides smaller. These data suggest that the 5'-UTR of the 5HT2a gene is capable of inhibiting translation and that this gene may utilize an alternate promoter to direct the transcription of a smaller, possibly better translated mRNA. (HD 05291 and HD 29951)

1861

THE SP1 SITES IN THE PROMOTER OF RECEPTOR FOR AGE (RAGE) ARE REQUIRED FOR INDUCION OF RAGE EXPRESSION IN NEURONAL CELLS BY AMPHOTERIN. J.F. Li, X.Q Qu and A. M. Schmidt (SPON: D. STERN). Columbia University Medical Center, New York, NY 10032

Enhanced expression of Receptor for AGE (RAGE) in the developing nervous system co-localizes with that of one of its ligands, amphoterin. In vitro, amphoterin-RAGE interaction mediates neurite outgrowth. To test regulation of RAGE expression, we incubated neuronal cells with amphoterin. RAGE expression increased ≈54 % and RAGE mRNA increased ≈2.1 fold, a process inhibited by anti-RAGE IgG. This was associated with an ≈2.3-fold increase in Sp1 by electrophoretic mobility shift assay (EMSA). To determine if Sp1 sites mediated enhanced expression of RAGE by amphoterin, we examined region (-420 to +45) of the RAGE gene. Two functional Sp1 sites were identified at -189/-180 and -45/-40 by DNase I footprinting and EMSA. Transient transfection of 5'-deletion RAGE reporter gene constructs into neuronal cells revealed that upon stimulation with amphoterin, ≈2.9-fold increased relative luciferase activity was demonstrated from constructs containing Sp1 sites vs. those without Sp1 sites. Both Sp1 sites were required; simultaneous mutation of two Sp1 sites led to complete loss of responsiveness to amphoterin. These data suggest that RAGE expression in neuronal development is mediated by amphoterin in an Sp1-dependent manner.

1862

Endothelin Receptor Is Down-regulated in SV40 Virus Transformed IMR90 and WI38 Cells. W.J. Chiou, J. Wang, L. J. Gehrke, C. E. Berg and J. R. Wu-Wong. Abbott Laboratories, Pharmaceutical Products Division, Abbott Park, IL 60064.

SV40 has been identified as one of the oncogenic DNA tumor viruses that induces malignant transformation by regulating some growth-related gene expressions, such as p53. Endothelin (ET) is a 21 amino acid peptide, which exerts its biological effects through binding to ET receptors. This report examines whether SV40 transformation would affect ET receptor expression. The results from binding and RT-PCR studies indicate that IMR90, a human lung fibroblast cell line, expresses predominantly type-A ET receptor (ET_A) and that ET receptor expression is significantly down-regulated in IMR90-SV40, a SV40 virus transformed IMR90 cell line. In a thymidine incorporation study, ET-1 stimulated DNA synthesis in IMR90 cells in serum-free medium. However, the proliferation of IMR90-SV40 is independent of fetal bovine serum or ET-1. Similar results are observed in the normal human lung fibroblast WI38 cells versus SV40 virus transformed WI38 cells. These results may have significant implications in understanding how SV40 virus regulates gene expression in cells.

1863

MIE BINDING PROTEINS ARE REQUIRED FOR 1,25-DIHYDROXYVITAMIN D_3 REGULATION OF *c-myc* DURING HL-60 CELL DIFFERENTIATION. Q. Pan and R.U. Simpson. Department of Pharmacology, University of Michigan Medical School, Ann Arbor, MI 48109.

Our laboratory showed that 1,25-dihydroxyvitamin D_3 [1,25-$(OH)_2D_3$] suppress *c-myc* expression during HL-60 cell differentiation by blocking transcriptional elongation at the first exon/intron border of the *c-myc* gene. In this study, we explored the functional importance of three putative regulatory protein binding sites found within a 280 bp region in intron 1 of the *c-myc* gene. HL-60 promyelocytic leukemia cells were transiently transfected with *c-myc* promoter constructs cloned upstream of a CAT reporter gene. With the wildtype *c-myc* promoter construct (pMPCAT), which contains MIE1, MIE2, and MIE3 sequences, 1,25-$(OH)_2D_3$ was able to decrease CAT activity by 43.2%. The ability of 1,25-$(OH)_2D_3$ to inhibit CAT activity was significantly decreased to 11.3% when examined with a MIE1 deletion construct (pMPCAT-MIE1) and completely blunted (-5.0%) when a 287 bp deletion construct (pMPCAT-287), without MIE1, MIE2, and MIE3 sequences, was analyzed. MIE1 and MIE2 binding proteins induced by 1,25-$(OH)_2D_3$ have similar gel shift mobilities, while MIE3 binding proteins migrated differently. Furthermore, chelerythrine chloride, a specific PKC inhibitor, inhibited nuclear proteins induced by 1,25-$(OH)_2D_3$ to bind to MIE1, MIE2, and MIE3. Taken together, our results suggest that regulation of *c-myc* transcription by 1,25-$(OH)_2D_3$ involves specific phosphorylated proteins binding to MIE1, MIE2 and/or MIE3 sequences.

1864

Endometrial cancer and APC gene mutations. R.Mituński[1], D.Lurnouf[2], M.Nothisen[2], J.Tomaszewski[1], J.Jakowicki[1], R.Fuchs[2]. 2nd Dep.Gynecol.Surg. Medical Academy, Lublin, Poland[1] Lab.Cancer.Mutag.Mol.Struct.,UPR 9003-CNRS,IRCAD, Strasbourg, France[2]

Introduction: APC is a suppressor gene for which inherited mutations predispose to colon cancer. One of the mark of mutated APC products is a defective activity in down-regulation of b-catenin-Tcf-4 transcriptional complex (Morin et al., 1997). Endometrial cancer presents similarities with colon cancer as arising from benign precursor lesions, same mutated genes as Ki-ras, p-53, DCC. Additionally, it was observed that endometrial cancer frequently occurs in women with colon cancer, however with non-polyposis type of colon cancer (Lynch II syndrom). **Material and Methods:** Therefore, our study was undertaken to evaluate if endometrial cancer patients have mutated APC suppressor gene. We tested 40 endometrial cancers and twelve normal endometrial tissues for mutation at nucleotide 1909-1911 of the APC gene within the sequence 5'-GTGGGAT-3' which has been shown to be a hot spot for PhIP induced (-G) frameshift mutagenesis. The technique used was based on the Mismatch Amplification Mutation Assay MAMA-PCR (Cha,1992) to selectively amplify mutated sequences using one of the primers with 3' end altered hybridization to wild or mutated APC sequence. The established sensitivity for this test is one mutated copy in 10^{-5} wild type copies to visualize PCR products with radioactive P^{32} after PAGE. **Results:** We found 7 tumors (17.5%) with mutation at investigated site of APC. Mostly, tumours were moderately or poorly differentiated (6 of 7), sharing different histopathology. We can conclude that APC suppressor gene is also mutated in human endometrial cancer as well as in colon cancer.

1865

SINGLE CELL ISOLATION AND GENE EXPRESSION ON MATERIALS WITH PATTERNED SURFACE CHEMISTRY

Carson H. Thomas, Charles S. Sfeir, Clive D. McFarland, and Kevin E. Healy. Northwestern University, Chicago, IL 60611 and CSIRO, Sydney, Australia 2113.

Performance of materials in biological environments (e.g., implants, biosensors) depends on the cell-surface interactions which take place immediately following exposure. The surface chemistry of these materials can be engineered to impart desired biological behavior, resulting in improved performance. Materials were designed to isolate either individual cells or small cell clusters (< 5 cells), and used to examine the influences of cell projected area on specific cell functions. By limiting the cell projected area differentiation and phenotypic expression can be manipulated. Surfaces were fabricated using a photolithographic process resulting in islands (ranging from 100μm^2 to 10,000μm^2) of an adhesive surface chemistry [N-(2-aminoethyl)-3-aminopropyl-trimethoxysilane: EDS] separated by a non-adhesive interpenetrating polymer network [poly acrylamide-co-ethylene glycol: P(AAm-co-EG)]. Determination of single cell protein expression on these surfaces required a reverse transcriptase in situ polymerase chain reaction be performed. Therefore, protein expression at the mRNA level could be observed within single cells with 56 different projected areas (>12,000 cells) on one surface. In particular, osteocalcin expression was examined within primary bone-derived cells exposed to these chemically patterned surfaces. These surfaces provide a useful tool for researching how cell morphology effects cell function, as well as, analysis of cell-material interactions. Supported by the Whitaker Foundation and NIH Grant NIAMS R01 AR43187 and NIDR T32 DE07042.

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292.9

BLOCKADE OF AMPHOTERIN RECEPTOR FOR AGE (RAGE) INTERACTION SUPPRESSES LUNG METASTASIS IN MURINE LEWIS LUNG CARCINOMA

A. Taguchi, D.C. Blood, G. del Toro, A. Lu, A. Canet, W. Qu and A.M. Schmidt (SPON: D.Stern) VF

Columbia University College of P&S, N.Y., NY 10032

The Receptor for AGE (RAGE) interacts with distinct ligands in development, its interaction with amphoterin, a polypeptide expressed in developing neurons, mediates neurite migration and outgrowth. We speculated that since amphoterin is re-expressed in transformed cells, that its interaction with tumor RAGE might mediate migration and invasion. We previously reported that transfection of C6 glioma cells with a construct encoding soluble RAGE (sRAGE), the extracellular ligand-binding domain of RAGE, or intraperitoneal administration of sRAGE in mice bearing C6 glioma, suppressed local growth and spread of tumors implanted in mice. Here, we tested these hypotheses in the Lewis lung carcinoma model of metastasis. Lewis lung carcinoma cells (2×10^6/0.1 ml) were injected into C57BL/6J mice. Two weeks later, tumors were completely excised. Three days prior to excision, mice were treated systemically with either daily murine sRAGE or murine serum albumin (MSA) control for three weeks. Upon sacrifice, lungs were examined for evidence of metastases. The mean ± SE of pleural surface metastases was: murine serum albumin 100 µg, 8.7 ± 1.4; sRAGE 2 µg, 4.6 ± 1.0 ($p=0.02$ compared to MSA); sRAGE 20 µg, 2.2 ± 1 ($p=0.001$ compared to MSA); and sRAGE 100 µg, 1.0 ± 0.3 metastases ($p<0.0001$ compared to MSA). Histologic examination of lungs obtained from sRAGE (100 µg) vs. MSA treated mice revealed a striking decrease in number/size of parenchymal metastases. In *in vitro* assays, inclusion of either sRAGE, anti-RAGE IgG or anti-amphoterin IgG markedly suppressed migration of either C6 glioma or Lewis lung carcinoma cells in Transwell dishes. In contrast, inclusion of MSA or nonimmune IgG was without effect. These data implicate amphoterin-RAGE interaction in tumor migration, local spread and metastasis. This interaction may represent a novel anti-tumor strategy.

292.10

THE PROGNOSTIC VALUE OF BETA 1-6 BRANCHED OLIGOSACCHARIDES IN HUMAN COLORECTAL CARCINOMA

W.K.F. Seelentag, W.P. Li, S.F. Hau Schmitz, U. Metzger, P. Aeberhard, P.U. Heitz and J. Rüth. Dept. of Pathol., Univ. of Zurich, CH-8091 Zürich and Swiss Group for Clinical Cancer Research, CH-3003 Bern, Switzerland

Increase of β1,6 branched oligosaccharides of glycoproteins is possibly associated with tumor progression and lymph node metastasis. The aim of this study was to determine the prognostic value of β1,6 branches in human colorectal carcinoma. Expression of β1,6 branches was histochemically evaluated using the leukoagglutinating Phaseolus vulgaris lectin, PHA-L, in tissue sections of 92 clinically documented colorectal carcinomas, of which 31 had formed lymph node metastases. The follow-up time ranged between 4 and 14 years (median 10.3 years). A PHA-L staining index taking into account staining intensity and its percentage of tumor cut surface area was established. The carcinoma staining index was highly associated with the disease free survival ($p = 0.004$) and overall survival ($p = 0.005$). Patients with a carcinoma staining index >1, as compared to those with a staining index ≤ 1, were at significantly higher risk for tumor recurrence with a shorter disease free survival (hazard ratio = 2.59; $p = 0.005$) and significant higher risk of death with shorter overall survival (hazard ratio 2.51; $p = 0.007$). The carcinoma staining index was also associated with the presence of lymph node metastases. We conclude that PHA-L staining in human colorectal carcinoma sections provides an independent prognostic indicator for tumor recurrence and patient survival and is associated with the presence of lymph node metastases.

292.11

Utility of Calretinin and HBME-1 Immunostaining in Differentiating Metastaic Renal Cell Carcinoma from Malignant Mesotheliomas: An Immunohistochemical Study.

A.Sharma, M.Rodriguez, L.T.Truong, P.T.Cagle. Baylor College of Medicine and The Methodist Hospital, Houston, TX

Aim: A considerable overlap exists between the histologic, immunophenotypic and ultrastructural characteristics of renal cell carcinomas (RCC) and malignant mesotheliomas (MM). A relative paucity of renal cell-specific and mesothelial-cell specific immunohistochemical markers further compromises a definitive differentiation. In this study we evaluated the differential expression of calretinin (cal) and HBME-1 in primary RCC, metastatic RCC and MM. **Design:** Paraffin-embedded tissue sections of 24 primary RCCs, 22 MMs and 11 RCCs metastatic to lung and pleura were immunostained for HBME-1 (1:50, Dako) and calretinin (1:200, Chemicon) using an ABC technique. Tumors were graded as negative (<5% positive cells) or positive. **Results:** Twenty-two of 24 (91%) primary RCCs showed no reactivity to either calretinin or HBME-1. Fifteen of 22 cases of MMs (86%) were positive for both antibodies. One (1/24) case of RCC, 2/11 of met. RCCs and 18/22 cases of MMs were calretinin positive. HBME-1 reactivity was seen in 1/24 RCCs, 2/11 met. RCCs and 16/22 MMs. Correlative expression of the antibodies is as follows:

Cal(-)/HBME-1(-):22 RCC/ 2 Met RCC/3 MM
Cal(+)/HBME-1(-): 1 RCC/7 Met RCC/3 MM
Cal(-)/HBME-1(+): 1 RCC/1 Met RCC/ 1 MM
Cal(+)/HBME-1(+): 0 RCC/1 Met RCC/15 MM

Statistical significance was reached using a Pearson chi2 analysis. Conclusion: The distinct calretinin/HBME-1 immunoprofile observed in MM and RCC is of diagnostic significance. However, a heterogeneous immunophenotypic expression in metastatic RCCs may indicate an altered biologic behavior.

CELLULAR SIGNAL TRANSDUCTION AND GENE EXPRESSION (293.1-293.2)

293.1

THE KIDNEY-EXPRESSED WINGED HELIX TRANSCRIPTION FACTOR FREAC-4 IS REGULATED BY ETS-1; A POSSIBLE ROLE IN KIDNEY DEVELOPMENT.

A. Cederberg, M. Hulander, P. Carlsson and S. Enerbäck

Dept. of Mol. Biol., Göteborg Univ., S-405 30 Göteborg, Sweden

FREAC-4 is a member of the forkhead family of winged helix transcriptional regulators. The kidney-expressed winged helix transcription factor FREAC-4 is regulated by Ets-1, another kidney-expressed transcription factor. Through transfection experiments three Ets-1 *cis*-elements are identified within the first 152 nucleotides upstream of the transcription start in the FREAC-4 promoter. These sites are confirmed in a DNase I *in vitro* protection assay using recombinant Ets-1 protein. In cotransfection experiments, using an Ets-1 expression vector, the induction of FREAC-4 reporter gene activity is attenuated approximately 6-fold when the three Ets-1 binding sites are mutated. Furthermore, we demonstrate that over-expression of Ets-1 in the human embryonic kidney cell line 293 is sufficient to increase FREAC-4 mRNA levels. These results are compatible with Ets-1 as an upstream regulator of FREAC-4 expression during kidney development.

This work was supported with grants from The Swedish Medical Research Council (MFR).

293.2

THE WINGED HELIX TRANSCRIPTION FACTOR FREAC-6 IS REQUIRED FOR NORMAL DEVELOPMENT OF THE INNER EAR

M. Hulander, W. Wurst, P. Carlsson & S. Enerbäck

Dept of Mol.Biol., Göteborg Univ, S-405 30, Sweden and GSF-Cent., Oberschleisheim, D-85764, Germany

Freac-6 is a member of the forkhead family of winged helix transcriptional regulators. Forkhead genes have previously been shown to be instrumental during embryogenesis in mammals, in particular during development of the nervous system. Mice with a targeted disruption of the *freac-6* locus exhibit circling behaviour, pathological swim test and abnormal reaching response – all common findings in mice with vestibular dysfunction. These animals also fail to elicit a Preyer reflex, in response to a suprathreshold auditory stimulation, as seen in mice with profound hearing impairment. Histological examination of the inner ear reveals a gross structural malformation of the vestibular part of the inner ear as well as the cochlea. These structures have been replaced by a single irregular cavity in which neither proper semicircular ducts nor cochlea can be identified. We also show that at day 9.5 post coitum (p.c.) *freac-6* is exclusively expressed in the otic vesicle. These findings, implicate *freac-6* as an early regulator necessary for development of both cochlea and vestibulum, and identify a previously unknown candidate deafness gene.

This work was supported with grants from The Swedish Medical Research Council.

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EXPRESSION OF RECEPTORS FOR ADVANCED GLYCOSYLATION END PRODUCTS ON RENAL CELL CARCINOMA CELLS IN VITRO

Shunji Miki, Soji Kasayama*, Yoshitsugu Miki**, Yu Nakamura, Masahiro Yamamoto, Bunzo Sato* and Tadamitsu Kishimoto*

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Proteins that have been modified by long-term expose to glucose accumulate advanced glycosylation end products (AGEs) as a function of protein age. In these studies, we have examined the interaction of AGE-protein with renal cell carcinoma cells (RCC) in vitro, using AGE-modified bovine serum albumin (AGE-BSA) as a probe. AGE-BSA showed tendency to induce in vitro cell growth of RCC cells and promoted the production of interleukin-6 (IL-6), an in vitro autocrine growth factor. Reverse transcriptase-polymerase chain reaction analysis revealed that RCC cells used here express mRNA for a receptor for AGEs(RAGE). These results suggested that AGEs taken up through RAGE on RCC cells might play a role in promoting the growth of RCC cells.

Epidemiologic data showed that diabetes mellitus was common in patients with renal cell carcinoma (RCC) (1). However, the molecular explanation of this association remains totally unclear. We supposed that hyperglycemia associated with diabetes mellitus might be involved in the development of RCC. One of the consequences of hyperglycemia is the formation of advanced glycosylation end products (AGEs), which result from a series of rearrangements secondary to nonenzymatic reaction of glucose with proteins (2).

AGEs were shown to be taken up through AGE specific receptors which are distinct from mannose-fucose receptors involved in glycoprotein uptake and from scavenger receptors, such as those for modified LDL and formaldehyde-treated albumin (2). The binding of AGEs to AGE specific receptors on

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macrophages have been shown to influence a broad range of cellular functions and to contribute to some of the principal features of vascular disease in the elderly and in people with diabetes (3). A receptor for AGEs, termed as RAGE, was initially isolated from bovine lung extract (4), which was subsequently identified in aortic endothelial cells (5) and macrophages (6).

The observation that fibroblasts and mesangial cells also express AGE receptors (7) led us to examine whether RCC cells also possess a similar mechanism for recognizing and processing AGEs.

In the present communication we showed the existence of RAGE on RCC cells and evaluated the effects of AGEs on the growth of RCC cells and on the production of IL-6 by RCC cells in vitro, using AGE-modified bovine serum albumin (BSA) as a probe.

MATERIALS AND METHODS

Cells: Clear cell type RCC cells were obtained from three patients as described previously (8). Cells were all positively stained with periodic acid-Schiff (PAS) and monoclonal anti-renal cell carcinoma antibody, K 2.7 (9). Freshly isolated RCC cells were cultured in Eagle MEM with 10% fetal calf serum (FCS)(Flow Laboratories,Inc.). RCC cells used in this experiment (a,b,c) have been demonstrated to have scavenger receptors (10).

Preparation of AGE-BSA: AGE-bovine serum albumin (BSA) was prepared by incubating BSA (essentially fatty acid free;Sigma) in phosphate-buffered saline (PBS) with 250mM glucose 6-phosphate (Glc-6-P) at 37°C for 8 wk in the presence of 1.5mM phenylmethylsulfonyl fluoride, 0.5mM EDTA, as described (11). Unincorporated glucose was removed by dialysis against 1 x PBS. Control BSA was exposed to 37°C for the same time interval and in the same buffer, except that Glc-6-P was omitted. The concentration of AGE-BSA or BSA was determined by the method of Bradford (12).

Determination of interleukin-6 (IL-6): IL-6 was determined by enzyme-linked immunosorbent assay (ELISA) with anti-human IL-6 monoclonal antibody and goat anti-human IL-6 polyclonal antibody, as described previously (10).

Oligonucleotides used for amplification: The sequences of oligonucleotide primers were designed based on human RAGE sequence (4); CACCTTCTCCTGTAGCTTCA (5' primer), TGCCACAAGATGACCCCAAT (3' primer). The predicted length of the RAGE amplification product is 480 bases.

Amplification of reverse-transcribed RNA by PCR: Total RNA from RCC cells and human umbilical vein endothelial cells was extracted with guanidium thiocyanate/ phenol/ chloroform (13). RNA was reverse-transcribed into cDNA by incubating 10 μg of total RNA with the first standard buffer (50 mM Tris-HCl, pH 8.3/ 75 mM KCl/ 3 mM $MgCl_2$), 10 mM DTT, 0.5 mM dNTP, 1.25 units/ ml oligo $(dT)^{12-18}$, 100 μg/ml BSA, 4 units/μl RNase inhibitor and 20 units/μl Moloney murine leukemia virus reverse transcriptase (GIBCO BRL, Gaithersburg, MD) at 37°C for 60 min. PCR was carried out at a concentration of 1 x PCR buffer, 0.2 mM dNTP, 0.8 μM each 5' and 3' primers, and 0.025 units/μl thermostable Taq polymerase (Perkin-Elmer Cetus, Norwalk, CT). The

amplification profile involved a denaturation step at 96°C for 1 min, annealing at 55°C for 45 sec, and extension at 72°C for 2 min for 30 cycles. The PCR products were electrophoresed in 1.5% agarose gels, and then the gels were stained with ethidium bromide.

RESULTS AND DISCUSSION

Renal cell carcinoma (RCC) cells freshly isolated from patients (a,b,c) were found not to grow in Eagle MEM medium without FCS. Normal (unmodified) BSA could induce *in vitro* cell growth of RCC cells in the abscence of FCS in a dose-dependent manner. It was examined whether AGE-BSA can induce *in vitro* cell growth of RCC cells compared with normal BSA. The results are shown in Fig.1(A). AGE-BSA showed tendency to induce *in vitro* cell growth of RCC cells compared with normal BSA.

We next examined the effects of AGE-BSA on the production of IL-6, which was proved to be an *in vitro* autocrine growth factor for RCC cells (8), by RCC cells (a,b,c). Conditioned media from RCC cells incubated in the abscence of FCS contained a little amount of IL-6. Normal BSA induced the production of IL-6 by RCC cells in the abscence of FCS in a dose-dependent manner. The amount of IL-6 elaborated after stimulation with AGE-BSA was obviously more than that induced in response to normal BSA [Fig.1 (B)]. Among the RCC cells examined, their ability to induce IL-6 was positively correlated with their degree of AGE-BSA-enhanced proliferation.

To confirm the existence of RAGE in RCC cells, we performed reverse transcriptase (RT)-PCR analysis using specific primers for human RAGE. mRNA expression of RAGE was detected in RNA preparation isolated from RCC cells (a,b,c) cultured in Eagle MEM medium with 10% FCS, as well as human umbilical vein endothelial cells as a positive control(Fig.2). An arrow shows the 480 base pair RAGE PCR product. PCR reactions without reverse transcriptase treatment resulted in disappearance of the PCR product (graphic data not shown). Thus, it is specific for RNA but not for contaminated DNA. In addition, cleavage of RT-PCR products with restriction enzymes generated the fragments expected from the previously reported sequence of human RAGE (4) (data not shown).

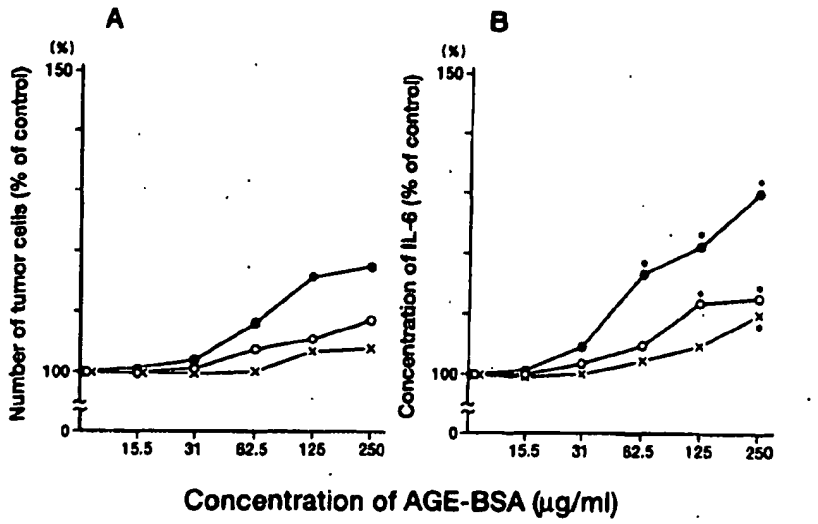

Fig.1. Effect of AGE-BSA compared with BSA on the cell growth of renal cell carcinoma cells and on the production of IL-6 in renal cell carcinoma cells.

(A) Tumor cells isolated from three patients [● (a), O (b), × (c)] were cultured (5×10^3 cells/well) in serum-free Eagle MEM with various concentrations of AGE-BSA or BSA in a 96 well microtiter plate (Nippon Becton Dickinson Co.). After 3 days, the number of recovered cells was determined. Number of tumor cells (% of control) was calculated by the formula: % of control = (the number of recovered cells with various concentrations of AGE-BSA / the number of recovered cells with various concentrations of BSA) × 100. The numbers of recovered cells with 250μg/ml of BSA were $6.2 \pm 0.5 \times 10^3$ [● (a)], $5.8 \pm 0.6 \times 10^3$ [O (b)] and $5.2 \pm 0.3 \times 10^3$ [× (c)], respectively. Data represent the mean of triplicate cultures.

(B) Tumor cells isolated from three patients [● (a), O (b), × (c)] were cultured (1×10^5 cells/ml) in serum-free Eagle MEM with various concentrations of AGE-BSA or BSA in a 24 well microtiter plate. After 6 hours, supernatants were then harvested and IL-6 was determined. Concentration of IL-6 (% of control) was calculated by the formula: % of control = (concentration of IL-6 in the supernatant derived from renal cell carcinoma cells cultured with various concentrations of AGE-BSA / concentration of IL-6 in the supernatant derived from renal cell carcinoma cells cultured with various concentrations of BSA) × 100. The concentrations of IL-6 in the supernatant derived from renal cell carcinoma cells cultured with 250μg/ml of BSA were 410±24 pg/ml [● (a)], 362±31 pg/ml [O (b)] and 227±19 pg/ml [× (c)], respectively. Data represent the mean of triplicate cultures. * $p < 0.05$ vs control values.

These experiments suggested that AGE might play a role in promoting the growth of RCC cells.

We have recently demonstrated that modified LDL taken up through scavenger receptor, which was thought to be expressed on macrophages in the atherosclerotic region, on RCC cells promoted the production of IL-6 to proliferate the cells (10).

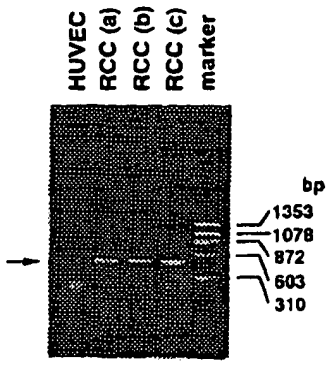

Fig.2. Identification of human receptor for advanced glycosylation end products (RAGE) mRNA in RCC cells by RT-PCR. RCC cells were isolated from three patients [(a),(b),(c)]. The predicted length of PCR product (480bp) was shown by an arrow. HUVEC, human umbilical vein endothelial cells.

Previous works have demonstrated that macrophages, upon interaction with AGEs, were induced the release of cytokines such as TNFα, IL-1β (3), PDGF (14), and IGF-1 (15) which were potent progression factors for fibroblasts, endothelial cells and smooth muscle cells. On the other hand, it was also demonstrated that modified LDL taken up by macrophages through scavenger receptors initiated production of growth factors and cytokines such as TNFα (16) and IL-1β(17), resulting in foam cell formation and atherogenesis.

These pilot studies introduced the possibility that AGEs and modified LDL might play a significant role in the excessive proliferative changes common in a variety of diabetic and aging tissues.

In our experiments, it was shown that RCC cells cultured in vitro might proliferate or produce an autocrine growth factor by taking up chemically modified products such as AGE-BSA and acetyl-LDL through RAGE and scavenger receptor, respectively. To our knowledge, this is the first report to show that some cancer cells express RAGE and induce an autocrine growth factor in response to AGEs.

Epidemiologic data showed that the incidence of RCC was high in patients undertaking hemodialysis whose serum levels of AGEs (18) and LDL (19) were increased.

Thus, senescent macromolecules such as AGEs, nonenzymatic glycosylation products and modified LDL produced by free radical-initiated LDL peroxidation might contribute to the development of the growth of tumor cells as well as atherosclerosis in vivo.

The existence of RAGE on RCC cells and the effects of AGEs on RCC cells in vivo are unknown and further studies are necessary to clarify the mechanisms of the growth of RCC.

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